WEST

End of Result Set

Generate Collection

L3: Entry 1 of 1 File: USPT Nov 9, 1999

US-PAT-NO: 5981200
DOCUMENT-IDENTIFIER: US 5981200 A

TITLE: Tandem fluorescent protein constructs

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Heim; Roger	Del Mar	CA		
Cubitt; Andrew	San Diego	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The Regents of the University of California	Oakland	CA			02
Aurora Biosciences Corporation	La Jolla	CA			02

APPL-NO: 8/ 792553
DATE FILED: January 31, 1997

PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 08/594,575, filed Jan. 31, 1996.

INT-CL: [6] G01N 33/573, G01N 33/53, G01N 33/52, C12N 15/62
US-CL-ISSUED: 435/7.4; 435/7.72, 435/320.1, 435/325, 435/69.7, 435/183, 435/212, 530/350, 530/402, 536/23.4
US-CL-CURRENT: 435/7.4; 435/183, 435/212, 435/320.1, 435/325, 435/69.7, 435/7.72, 530/350, 530/402, 536/23.4
FIELD-OF-SEARCH: 435/6, 435/7.2, 435/7.21, 435/7.37, 435/7.4, 435/7.71, 435/7.72, 435/69.7, 435/183, 435/212, 435/252.3, 435/252.33, 435/320.1, 435/325, 530/350, 530/402, 536/23.4, 536/24.1, 930/280, 930/310

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
[ ]	4314936	February 1982	Yaron et al.	530/331
[ ]	5264563	November 1993	Huse	536/25.3
[ ]	5491084	February 1996	Chalfie	438/189
[ ]	5599906	February 1997	Dasmahapatra	530/350
[ ]	5602021	February 1997	Davis et al.	435/219
[ ]	5605809	February 1997	Komoriya et al.	435/23
[ ]	5614191	March 1997	Puri et al.	424/178.1
[ ]	5625048	April 1997	Tsien et al.	536/23.4

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 428 000 A1	May 1991	EPX	
WO 91/01305	February 1991	WOX	
WO 94/28166	August 1994	WOX	
94/28166	December 1994	WOX	
94/28173	December 1994	WOX	
WO 95/07463	March 1995	WOX	
WO 95/21191	August 1995	WOX	
96/13607	May 1996	WOX	
96/23810	August 1996	WOX	
96/23898	August 1996	WOX	
96/27027	September 1996	WOX	
96/27675	September 1996	WOX	
WO 97/11094	March 1997	WOX	
WO 97/28261	August 1997	WOX	

ART-UNIT: 166
PRIMARY-EXAMINER: Feisee; Lila
ASSISTANT-EXAMINER: Pak; Michael
ATTY-AGENT-FIRM: Fish & Richardson P.C.

ABSTRACT:

This invention provides tandem fluorescent protein construct including a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties. The donor and acceptor moieties exhibit fluorescence resonance energy transfer which is eliminated upon cleavage. The constructs are useful in enzymatic assays.

27 Claims, 10 Drawing figures

WEST

End of Result Set

Generate Collection

L3: Entry 1 of 1 File: USPT Nov 9, 1999

US-PAT-NO: 5981200
DOCUMENT-IDENTIFIER: US 5981200 A

TITLE: Tandem fluorescent protein constructs

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Heim; Roger	Del Mar	CA		
Cubitt; Andrew	San Diego	CA		

US-CL-CURRENT: 435/7.4; 435/183, 435/212, 435/320.1, 435/325, 435/69.7, 435/7.72, 530/350, 530/402, 536/23.4

CLAIMS:

What is claimed is:
1. A tandem fluorescent protein construct, comprising:
a donor fluorescent protein moiety,
an acceptor fluorescent protein moiety, and
a peptide linker moiety coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein cyclized amino acids of said donor fluorescent protein moiety emit light characteristic of said donor fluorescent protein moiety,
further wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said linker moiety does not substantially emit light to excite said donor fluorescent protein moiety.
2. The tandem fluorescent protein construct of claim 1, wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212.
3. The tandem fluorescent protein construct of claim 1, wherein both of said donor fluorescent protein moiety and said acceptor fluorescent protein moiety are an Aequorea-related fluorescent protein moiety.
4. The tandem fluorescent protein construct of claim 3, wherein said mutation is selected from the group consisting of F64L, S65G, S65T, Y66F, Y66H, Y66W, V68L, S72A, Y145F, N146I, N149K, M153T, V163A, I167T, T203I, T203Y, and N212K.
5. The tandem fluorescent protein construct of claim 4, wherein said mutation is selected for 10c, W1B, Emerald, and Sapphire.
6. The tandem fluorescent protein construct of claim 5, wherein said donor fluorescent protein moiety is P4-3, Sapphire, W7, or Y66H and said acceptor protein fluorescent moiety is W7, Topaz, S65T, or S65C, with the proviso that when said donor fluorescent protein moiety is P4-3, then said acceptor fluorescent protein moiety is not S65T or S65C and when said donor fluorescent protein moiety is W7, then said acceptor fluorescent moiety is not S65T.
7. The tandem fluorescent protein construct of claim 1, wherein said linker moiety comprises a cleavage recognition site for an enzyme.
8. The tandem fluorescent protein construct of claim 7, wherein said linker

moiety is a peptide moiety.
9. The tandem fluorescent protein construct of claim 8, wherein said donor fluorescent protein moiety, said acceptor fluorescent protein moiety, and said linker moiety comprise a single polypeptide.
10. The tandem fluorescent protein construct of claim 9, wherein said linker moiety comprises between about 5 and 50 amino acids.
11. The tandem fluorescent protein construct of claim 10, wherein said linker moiety comprises between about 10 and 30 amino acids.
12. The tandem fluorescent protein construct of claim 9, wherein said linker moiety comprises a cleavage recognition site for an enzyme selected from the group consisting of trypsin, enterokinase, HIV-1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, .beta.-secretase for amyloid precursor protein, thrombin, renin, angiotensin-converting enzyme, cathepsin D and a kininogenase.
13. The tandem fluorescent protein construct of claim 10, wherein said donor fluorescent protein moiety is positioned at the amino terminus of the polypeptide relative to said acceptor fluorescent protein moiety.
14. The tandem fluorescent protein construct of claim 1, wherein said peptide linker moiety is of a length and orientation that allows fluorescent energy transfer between said donor fluorescent protein moiety and said acceptor fluorescent protein moiety.
15. The tandem fluorescent protein construct of claim 7, comprising a cleavage recognition site for beta-lactamase.
16. The tandem fluorescent protein construct of claim 11, wherein said linker moiety comprises a protease recognition site.
17. A recombinant nucleic acid encoding for the expression of a functional tandem fluorescent protein construct, said tandem fluorescent protein construct comprising:
a donor fluorescent protein moiety,
an acceptor fluorescent protein moiety, and
a peptide linker moiety coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said peptide linker moiety does not substantially emit light to excite said donor fluorescent protein moiety, further wherein said peptide linker moiety comprises a cleavage recognition site for a protease.
18. The recombinant nucleic acid of claim 17, wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212.
19. The recombinant nucleic acid of claim 7, wherein said peptide linker moiety is of a length and orientation that allows fluorescent resonance energy transfer between said donor fluorescent protein moiety and said acceptor fluorescent protein moiety.
20. An expression vector, comprising: an expression control sequence operatively linked to a sequence coding for the expression of a functional tandem fluorescent protein construct, said tandem fluorescent protein construct comprising:
a donor fluorescent protein moiety,
an acceptor fluorescent protein moiety, and
a peptide linker moiety coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said peptide linker moiety does not substantially emit light to excite said donor fluorescent protein moiety further wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212.
21. A host cell transfected with an expression vector, said expression vector comprising: an expression control sequence operatively linked to a sequence coding for the expression of a functional tandem fluorescent protein construct,

said tandem fluorescent protein construct comprising:
a donor fluorescent protein moiety,
an acceptor fluorescent protein moiety, and
a peptide linker moiety coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said peptide linker moiety does not substantially emit light to excite said donor fluorescent protein moiety,
wherein said peptide linker moiety comprises a cleavage recognition site for a protease.

22. The host cell of claim 21, wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212.

23. A method for determining whether a sample contains an enzyme, comprising:
contacting a sample with a tandem fluorescent protein construct, said tandem fluorescent protein construct comprising:
a donor fluorescent protein moiety,
an acceptor fluorescent protein moiety, and
a linker moiety comprising a cleavage recognition site for an enzyme, coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein cyclized amino acids of said donor fluorescent protein moiety emit light characteristic of said donor fluorescent protein moiety,
further wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said linker moiety does not substantially emit light to excite said donor fluorescent protein moiety,
further wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212,
exciting said donor fluorescent protein moiety, and
determining a fluorescence property in said sample,
wherein the presence of said enzyme in said sample results in a change in the degree of fluorescence resonance energy transfer.

24. A method for determining the activity of an enzyme in a cell, comprising:
providing a cell that expresses a tandem fluorescent protein construct, said tandem fluorescent protein construct comprising:
a donor fluorescent protein moiety,
an acceptor fluorescent protein moiety, and
a peptide linker moiety comprising a cleavage recognition amino acid sequence specific for said enzyme coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein cyclized amino acids of said donor fluorescent protein moiety emit light characteristic of said donor fluorescent protein moiety,
further wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said linker moiety does not substantially emit light to excite said donor fluorescent protein moiety,
further wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212,
exciting said donor fluorescent protein moiety, and
determining the degree of fluorescence resonance energy transfer in said cell,
wherein the presence of said activity in said cell results in a change in the degree of fluorescence resonance energy transfer.

25. A method for determining the amount of activity of an enzyme in a sample from an organism, comprising:
contacting a sample from an organism with a tandem fluorescent protein construct, said construct comprising
a donor fluorescent protein moiety,

an acceptor fluorescent protein moiety, and
a peptide linker moiety comprising a cleavage recognition amino acid sequence specific for said enzyme coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein cyclized amino acids of said donor fluorescent protein moiety emit light characteristic of said donor fluorescent protein moiety,
further wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said peptide linker moiety does not substantially emit light to excite said donor fluorescent protein moiety,
further wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212, and
exciting said donor fluorescent protein moiety, and
determining the degree of fluorescence resonance energy transfer in said sample, wherein the presence of said activity in said sample results in a change in the degree of fluorescence resonance energy transfer.

26. A method for determining whether a compound alters the activity of an enzyme, comprising:
contacting a sample containing an enzyme with a compound and a tandem fluorescent protein construct, said tandem fluorescent protein construct comprising:
a donor fluorescent protein moiety,
an acceptor fluorescent protein moiety, and
a linker moiety coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein cyclized amino acids of said donor fluorescent protein moiety emit light characteristic of said donor fluorescent protein moiety,
further wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said linker moiety does not substantially emit light to excite said donor fluorescent protein moiety,
further wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212, and
exciting said donor fluorescent protein moiety, and
determining a fluorescent property of said sample,
wherein an activity of said enzyme is determined by a change in the degree of said fluorescent property in the presence and absence of said compound.

27. A method for determining whether a compound alters the activity of an enzyme in a cell, comprising:
providing a first and second cells that express a tandem fluorescent protein construct, said tandem fluorescent protein construct comprising:
a donor fluorescent protein moiety,
an acceptor fluorescent protein moiety, and
a peptide linker moiety comprising a cleavage recognition amino acid sequence specific for said enzyme coupling said donor fluorescent protein moiety and said acceptor fluorescent protein moiety,
wherein cyclized amino acids of said donor fluorescent protein moiety emit light characteristic of said donor fluorescent protein moiety,
further wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited, and said peptide linker moiety does not substantially emit light to excite said donor fluorescent protein moiety, and
exciting said donor fluorescent protein moiety,
contacting said first cell with an amount of said compound, contacting the second cell with a different amount of said compound,
further wherein at least one of said donor fluorescent moiety and said acceptor fluorescent protein moiety comprises an Aequorea-related fluorescent protein moiety comprising a mutation at a position selected from the group consisting of F64, S65, Y66, V68, S72, Y145, N146, N149, M153, V163, I167, T203, and N212, and
exciting said donor fluorescent protein moiety in said first and second cell,

determining the degree of fluorescence resonance energy transfer in said first and second cells, and

comparing the degree of fluorescence resonance energy transfer in said first cell and said second cell, wherein a difference in the degree of fluorescence resonance energy transfer in said first cell and said second cell indicates that the compound alters the activity of said enzyme.

Trying 3106016892...Open

Welcome to STN International! Enter x:x
LOGINID:ssspta1800exs
PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Dec 17	The CA Lexicon available in the CAPLUS and CA files
NEWS 3	Feb 06	Engineering Information Encompass files have new names
NEWS 4	Feb 16	TOXLINE no longer being updated
NEWS 5	Apr 23	Search Derwent WPINDEX by chemical structure
NEWS 6	Apr 23	PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
NEWS 7	May 07	DGENE Reload
NEWS 8	Jun 20	Published patent applications (A1) are now in USPATFULL
NEWS 9	JUL 13	New SDI alert frequency now available in Derwent's DWPI and DPCI
NEWS 10	Aug 23	In-process records and more frequent updates now in MEDLINE
NEWS 11	Aug 23	PAGE IMAGES FOR 1947-1966 RECORDS IN CAPLUS AND CA
NEWS 12	Aug 23	Adis Newsletters (ADISNEWS) now available on STN
NEWS 13	Sep 17	IMSworld Pharmaceutical Company Directory name change to PHARMASEARCH
NEWS 14	Oct 09	Korean abstracts now included in Derwent World Patents Index
NEWS 15	Oct 09	Number of Derwent World Patents Index updates increased
NEWS 16	Oct 15	Calculated properties now in the REGISTRY/ZREGISTRY File
NEWS 17	Oct 22	Over 1 million reactions added to CASREACT
NEWS 18	Oct 22	DGENE GETSIM has been improved
NEWS 19	Oct 29	AAASD no longer available
NEWS 20	Nov 19	New Search Capabilities USPATFULL and USPAT2
NEWS 21	Nov 19	TOXCENTER(SM) - new toxicology file now available on STN
NEWS 22	Nov 29	COPPERLIT now available on STN
NEWS 23	Nov 29	DWPI revisions to NTIS and US Provisional Numbers
NEWS 24	Nov 30	Files VETU and VETB to have open access
NEWS 25	Dec 10	WPINDEX/WPIDS/WPIX New and Revised Manual Codes for 2002
NEWS 26	Dec 10	DGENE BLAST Homology Search
NEWS 27	Dec 17	WELDASEARCH now available on STN
NEWS 28	Dec 17	STANDARDS now available on STN
NEWS 29	Dec 17	New fields for DPCI
NEWS 30	Dec 19	CAS Roles modified
NEWS 31	Dec 19	1907-1946 data and page images added to CA and CAplus

NEWS EXPRESS	August 15 CURRENT WINDOWS VERSION IS V6.0c, CURRENT MACINTOSH VERSION IS V6.0 (ENG) AND V6.0J (JP), AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

* * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 12:2[redacted] 08 JAN 2002

=> fil .eliz
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.15	0.15

FILE 'MEDLINE' ENTERED AT 12:22:04 ON 08 JAN 2002

FILE 'SCISEARCH' ENTERED AT 12:22:04 ON 08 JAN 2002
COPYRIGHT (C) 2002 Institute for Scientific Information (ISI) (R)

FILE 'LIFESCI' ENTERED AT 12:22:04 ON 08 JAN 2002
COPYRIGHT (C) 2002 Cambridge Scientific Abstracts (CSA)

FILE 'BIOTECHDS' ENTERED AT 12:22:04 ON 08 JAN 2002
COPYRIGHT (C) 2002 DERWENT INFORMATION LTD

FILE 'BIOSIS' ENTERED AT 12:22:04 ON 08 JAN 2002
COPYRIGHT (C) 2002 BIOSIS(R)

FILE 'EMBASE' ENTERED AT 12:22:04 ON 08 JAN 2002
COPYRIGHT (C) 2002 Elsevier Science B.V. All rights reserved.

FILE 'HCAPLUS' ENTERED AT 12:22:04 ON 08 JAN 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'NTIS' ENTERED AT 12:22:04 ON 08 JAN 2002
Compiled and distributed by the NTIS, U.S. Department of Commerce.
It contains copyrighted material.
All rights reserved. (2002)

FILE 'ESBIOBASE' ENTERED AT 12:22:04 ON 08 JAN 2002
COPYRIGHT (C) 2002 Elsevier Science B.V., Amsterdam. All rights reserved.

FILE 'BIOTECHNO' ENTERED AT 12:22:04 ON 08 JAN 2002
COPYRIGHT (C) 2002 Elsevier Science B.V., Amsterdam. All rights reserved.

FILE 'WPIDS' ENTERED AT 12:22:04 ON 08 JAN 2002
COPYRIGHT (C) 2002 DERWENT INFORMATION LTD

=> s (aequorea or gfp) and muta?
9 FILES SEARCHED...
L1 8324 (AEQUOREA OR GFP) AND MUTA?

=> s l1 and 65
L2 132 L1 AND 65

=> s l1 and 145
L3 34 L1 AND 145

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 11 DUP REM L3 (23 DUPLICATES REMOVED)

=> d 1-11

L4 ANSWER 1 OF 11 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:886535 HCAPLUS
DN 136:15227
TI Use of trans-complementary viral vectors, containing deletion of E1, E3 or E4 viral genes and nucleotide sequences for a tumor suppressor gene or suicide gene, in tumor regression
IN Ramsey, William J.; Higginbotham, James N.; Link, Charles J.
PA Human Gene Therapy Research Institute, USA
SO PCT Int. Appl., 86 pp.

. CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001092550	A2	20011206	WO 2001-US17524	20010531

PI WO 2001092550 A2 20011206
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
PRAI US 2000-208248 P 20000531

L4 ANSWER 2 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS
AN 2001:371611 BIOSIS
DN PREV200100371611
TI Second generation suicide gene therapy using Herpes Simplex Virus Thymidine Kinase **mutants** for advanced prostate cancer.
AU Pantuck, Allan J. (1); Matherly, Jamie (1); Zisman, Amnon (1); Wu, Lily (1); Belldegrun, Arie S. (1)
CS (1) Los Angeles, CA USA
SO Journal of Urology, (May, 2001) Vol. 165, No. 5 Supplement, pp. 291. print.
Meeting Info.: Annual Meeting of the American Urological Association, Inc. Anaheim, California, USA June 02-07, 2001
ISSN: 0022-5347.
DT Conference
LA English
SL English

L4 ANSWER 3 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
AN 2000:597892 SCISEARCH
GA The Genuine Article (R) Number: 340DJ
TI Functional expression and biophysical properties of polymorphic variants of the human gap junction protein connexin37
AU Kumari S S; Varadaraj K; Valiunas V; Ramanan S V; Christensen E A (Reprint); Beyer E C; Brink P R
CS SUNY STONY BROOK, DEPT PHYSIOL & BIOPHYS, STONY BROOK, NY 11794 (Reprint); SUNY STONY BROOK, DEPT PHYSIOL & BIOPHYS, STONY BROOK, NY 11794; UNIV CHICAGO, DEPT PEDIAT, CHICAGO, IL 60637
CYA USA
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (21 JUL 2000) Vol. 274, No. 1, pp. 216-224.
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0006-291X.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 43
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L4 ANSWER 4 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
AN 2000:366777 SCISEARCH
GA The Genuine Article (R) Number: 311VP
TI Molecular analysis of racE function in Dictyostelium
AU Larochelle D A (Reprint); Gerald N; DeLozanne A
CS CLARK UNIV, DEPT BIOL, 950 MAIN ST, WORCESTER, MA 01610 (Reprint); DUKE UNIV, MED CTR, DEPT CELL BIOL, DURHAM, NC 27710; UNIV TEXAS, SECT MOL CELL & DEV BIOL, AUSTIN, TX 78712
CYA USA
SO MICROSCOPY RESEARCH AND TECHNIQUE, (15 APR 2000) Vol. 49, No. 2, pp. 145-151.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK,

. NY 10158-0012.
ISSN: 1059-910X.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L4 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:718875 HCAPLUS
DN 131:348774
TI Tandem fluorescent protein constructs and their preparation for enzyme assays
IN Tsien, Roger Y.; Heim, Roger; Cubitt, Andrew
PA The Regents of the University of California, USA; Aurora Biosciences Corporation
SO U.S., 33 pp., Cont.-in-part of U.S. Ser. No. 594,575.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5981200	A	19991109	US 1997-792553	19970131
PRAI US 1996-594575		19960131		

RE.CNT 22
RE
(1) Anon; EP 0428000 A1 1991 HCAPLUS
(2) Anon; WO 9101305 1991 HCAPLUS
(3) Anon; WO 9428166 1994 HCAPLUS
(4) Anon; WO 9428166 1994 HCAPLUS
(5) Anon; WO 9428173 1994 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 11 MEDLINE DUPLICATE 1
AN 1999432217 MEDLINE
DN 99432217 PubMed ID: 10500161
TI Circular permutation and receptor insertion within green fluorescent proteins.
AU Baird G S; Zacharias D A; Tsien R Y
CS Department of Pharmacology, University of California at San Diego, La Jolla, CA 92093-0647, USA.
NC NS27177 (NINDS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Sep 28) 96 (20) 11241-6.
Journal code: PV3; 7505876. ISSN: 0027-8424.
not prior art
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199910
ED Entered STN: 19991101
Last Updated on STN: 19991101
Entered Medline: 19991021

L4 ANSWER 7 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
AN 1999:911727 SCISEARCH
GA The Genuine Article (R) Number: 256VB
TI Visualizing multiple constrictions in spheroidal Escherichia coli cells
AU Zaritsky A (Reprint); VanGeel A; Fishov I; Pas E; Einav M; Woldringh C L
CS BEN GURION UNIV NEGEV, DEPT LIFE SCI, POB 653, IL-84105 BEER SHEVA, ISRAEL (Reprint); UNIV AMSTERDAM, SECT MOL CYTOL, INST MOL CELL BIOL, BIOCENTRUM, NL-1098 SM AMSTERDAM, NETHERLANDS
CYA ISRAEL; NETHERLANDS
SO BIOCHIMIE, (AUG-SEP 1999) Vol. 81, No. 8-9, pp. 897-900.
Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER, 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE.
ISSN: 0300-9084.
DT Article; Journal

FS LIFE
LA English
REC Reference Count: 40
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L4 ANSWER 8 OF 11 MEDLINE DUPLICATE 2
AN 1998192613 MEDLINE
DN 98192613 PubMed ID: 9525926
TI Improved fluorescence and dual color detection with enhanced blue and green variants of the green fluorescent protein.
AU Yang T T; Sinai P; Green G; Kitts P A; Chen Y T; Lybarger L; Chervenak R; Patterson G H; Piston D W; Kain S R
CS Cell Biology Group, Clontech Laboratories, Inc., Palo Alto, California 94303, USA.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Apr 3) 273 (14) 8212-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199805
ED Entered STN: 19980514
Last Updated on STN: 19980514
Entered Medline: 19980507

This work

L4 ANSWER 9 OF 11 MEDLINE DUPLICATE 3
AN 1998320591 MEDLINE
DN 98320591 PubMed ID: 9647829
TI Use of green fluorescent protein to tag and investigate gene expression in marine bacteria.
AU Stretton S; Techkarnjanaruk S; McLennan A M; Goodman A E
CS School of Biological Sciences, Flinders University of South Australia, Adelaide, Australia.
SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1998 Jul) 64 (7) 2554-9.
Journal code: 6K6; 7605801. ISSN: 0099-2240.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199808
ED Entered STN: 19980820
Last Updated on STN: 19980820
Entered Medline: 19980811

L4 ANSWER 10 OF 11 BIOTECHDS COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 1996-12121 BIOTECHDS
TI New modified **Aequorea** green fluorescent polypeptides; green fluorescent protein engineering for altered excitation and/or emission spectrum, for use as a reporter gene
AU Tsien R Y; Heim R
PA Univ.California
LO Oakland, CA, USA.
PI WO 9623810 8 Aug 1996
AI WO 1995-US14692 13 Nov 1995
PRAI US 1994-337915 10 Nov 1994
DT Patent
LA English
OS WPI: 1996-371370 [37]

L4 ANSWER 11 OF 11 MEDLINE DUPLICATE 4
AN 96284100 MEDLINE
DN 96284100 PubMed ID: 8673464
TI Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence resonance energy transfer.
AU Heim R; Tsien R Y
CS Howard Hughes Medical Institute 0647, University of California, San Diego, La Jolla 92093-0647, USA.
SO CURRENT BIOLOGY, (1996 Feb 1) 6 (2) 178-82.
Journal code: B44; 9107782. ISSN: 0960-9822.

CY ENGLAND: United Kingdo[redacted]
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199608
ED Entered STN: 19960822
Last Updated on STN: 19980206
Entered Medline: 19960815

=> d 1-11 kwic

L4 ANSWER 1 OF 11 HCAPLUS COPYRIGHT 2002 ACS
AB . . . nucleotide sequences included within the said vector. The invention further relates that the first or second viral vector may contain **mutations** in the E1, E3 or E4 viral genes, and can include nucleotide sequences encoding heterologous proteins, such as green fluorescent protein (**GFP**), or nucleotide sequences for a tumor suppressor gene or suicide gene. The invention also provides transformation compns. comprising a mixt.. . . an agent, such as radioactive iodine. In the example section, the invention presents the construction of several viral vectors (Ad **GFP**, Ad dl1011, Ad dl1010, Ad dl1020) and shows their ability to transduce tumor cells (such as DU **145** cells). The invention also described the development of an animal model for adrenocortical carcinoma, i.e. SW-13-derived human adrenocortical carcinoma xenograft. . .
IT Reporter gene
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(**GFP**, for green fluorescent protein; use of trans-complementary viral vectors, contg. deletion of E1, E3 or E4 viral genes and nucleotide sequences for a tumor suppressor gene or suicide gene, in tumor regression)

L4 ANSWER 2 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS
TI Second generation suicide gene therapy using Herpes Simplex Virus Thymidine Kinase **mutants** for advanced prostate cancer.
ORGN . . .
Herpesviridae: Animal Viruses, Viruses, Microorganisms; Hominidae: Primates, Mammalia, Vertebrata, Chordata, Animalia; Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
CL1-**GFP** cell line (Hominidae): metastatic prostate cancer cell line; CMV [cytomegalovirus] (Herpesviridae); DU-**145** cell line (Hominidae): human prostate carcinoma cell; Herpes simplex virus (Herpesviridae): gene vector; LnCAP cell line (Hominidae): human prostate cancer. . .

L4 ANSWER 3 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
AB . . . to 300 pS). However, addition of an amino terminal T7 tag (T7-Cx37-fs254 Delta 293) produced a single channel conductance of 120-**145** pS with a 24-30 pS residual state. Moreover, the kinetics of the voltage-dependent decline in junctional current for T7-Cx37-fs254 Delta. . .
ST Author Keywords: intercellular communication; connexin; truncation; frame shift; **GFP** tag; T7 tag; conductance
STP KeyWords Plus (R): MARIE-TOOTH DISEASE; VASCULAR SMOOTH-MUSCLE; VOLTAGE DEPENDENCE; MOLECULAR ANALYSIS; **MUTATIONS**; CHANNELS; GENE; PERMEABILITY; CONDUCTANCE; MESSENGER

L4 ANSWER 4 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
AB . . . in cytokinesis. The racEgene was isolated in a screen designed to identify genes specifically required for cytokinesis. The use of **GFP** fusion proteins, coupled with **mutational** analysis, allowed us to determine that racE exerts its function at the plasma membrane throughout the entire cell cycle. Measurements. . . at the cortex. We postulate that in the absence of proper cortical tension, cytokinesis cannot proceed normally. Microsc. Res. Tech. 49:**145**-151, 2000. (C) 2000 Wiley-Liss, Inc.

L4 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2002 ACS
AB . . . and acceptor moieties exhibit fluorescence resonance energy transfer which is eliminated upon cleavage. The constructs are useful in enzymic assays. **Mutant** green fluorescent proteins (**GFPs**) were created by **mutagenesis** of the **Aequorea** victoria **GFP**. Polyhistidine tagged tandem green and blue fluorescent proteins were recombinantly constructed having an inserted peptide sequence including cleavage recognition sites. . .

IT **Aequorea** victoria
(constructs from **mutants** of fluorescent protein of; tandem fluorescent protein constructs and their prepn. for enzyme assays)

IT **Aequorea**
(fluorescent proteins of; tandem fluorescent protein constructs and their prepn. for enzyme assays)

IT **Mutagenesis**
(in **Aequorea**-related fluorescent proteins; tandem fluorescent protein constructs and their prepn. for enzyme assays)

IT 194370-56-4DP, Green fluorescent protein (**Aequorea** victoria), **mutants** and tandem dimers 194370-57-5P, Green fluorescent protein [66-histidine, **145**-phenylalanine] (**Aequorea** victoria) 194370-59-7P, Green fluorescent protein [65-threonine] (**Aequorea** victoria) 194370-60-0P, Green fluorescent protein [65-cysteine] (**Aequorea** victoria) 194370-61-1DP, Green fluorescent protein [64-leucine, 65-threonine, 66-tryptophan, 146-isoleucine, 153-threonine, 163-alanine, 212-lysine] (**Aequorea** victoria), tandem fluorescent protein construct contg. 194370-62-2P, Green fluorescent protein [65-threonine, 72-alanine, 149-lysine, 153-threonine, 167-threonine] (**Aequorea** victoria) 249590-48-5DP, tandem fluorescent protein construct contg. 249590-51-0P, PN: US5981200 TABLE: 1 claimed protein 249590-52-1P 249591-64-8P
RL: BPN (Biosynthetic preparation); BPR (Biological process); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)
(amino acid sequence; tandem fluorescent protein constructs and their prepn. for enzyme assays)

IT 203875-93-8P
RL: BPN (Biosynthetic preparation); BPR (Biological process); PRP (Properties); BIOL (Biological study); PREP (Preparation); PROC (Process)
(nucleotide sequence and **mutagenesis** of; tandem fluorescent protein constructs and their prepn. for enzyme assays)

L4 ANSWER 6 OF 11 MEDLINE DUPLICATE 1
AB Many areas of biology and biotechnology have been revolutionized by the ability to label proteins genetically by fusion to the **Aequorea** green fluorescent protein (**GFP**). In previous fusions, the **GFP** has been treated as an indivisible entity, usually appended to the amino or carboxyl terminus of the host protein, occasionally. . . tightly interwoven, three-dimensional structure and intricate posttranslational self-modification required for chromophore formation would suggest that major rearrangements or insertions within **GFP** would prevent fluorescence. However, we now show that several rearrangements of **GFPs**, in which the amino and carboxyl portions are interchanged and rejoined with a short spacer connecting the original termini, still. . . permutations have altered pKa values and orientations of the chromophore with respect to a fusion partner. Furthermore, certain locations within **GFP** tolerate insertion of entire proteins, and conformational changes in the insert can have profound effects on the fluorescence. For example, insertions of calmodulin or a zinc finger domain in place of Tyr-**145** of a yellow **mutant** (enhanced yellow fluorescent protein) of **GFP** result in indicator proteins whose fluorescence can be enhanced severalfold upon metal binding. The calmodulin graft into enhanced yellow fluorescent protein can monitor cytosolic Ca(2+) in single mammalian cells. The tolerance of **GFPs** for circular permutations and insertions shows the folding process is surprisingly robust and offers a new strategy for creating genetically. . .

L4 ANSWER 7 OF 11 SCISEARCH COPYRIGHT 2002 ISI (R)
AB . . . rate. Such a procedure has recently been performed by

thymine-limitation of [redacted] K12 strain CR34 (Zaritsky [redacted], Microbiology **145** (1999), 1021-1022). Enhancing the replication rate in cells with multi-forked replicating chromosomes (by addition of deoxyguanosine) shortens the intervals between. . . required to complete the division process in wide cells with long circumferences, observed during thymine step-up. Overexpression of fusion protein FtsZ-**GFP** on a multi-copy plasmid should circumvent the shortage. (C) 1999 Societe francaise de biochimie et biologic moleculaire/Editions scientifiques et medicales. . .

STP KeyWords Plus (R): PENICILLIN-BINDING PROTEINS; DIVISION CYCLE; CHROMOSOME REPLICATION; FTSZ; GROWTH; **MUTANT**; MORPHOLOGY; LENGTH; SHAPE

L4 ANSWER 8 OF 11 MEDLINE DUPLICATE 2

AB The green fluorescent protein (**GFP**) from the jellyfish **Aequorea** victoria is a versatile reporter protein for monitoring gene expression and protein localization in a variety of systems. Applications using **GFP** reporters have expanded greatly due to the availability of **mutants** with altered spectral properties, including several blue emission variants, all of which contain the single point **mutation** Tyr-66 to His in the chromophore region of the protein. However, previously described "BFP" reporters have limited utility, primarily due. . . expression levels attained in higher eukaryotes with such variants. To improve upon these qualities, we have combined a blue emission **mutant** of **GFP** containing four point **mutations** (Phe-64 to Leu, Ser-65 to Thr, Tyr-66 to His, and Tyr-**145** to Phe) with a synthetic gene sequence containing codons preferentially found in highly expressed human proteins. These **mutations** were chosen to optimize expression of properly folded fluorescent protein in mammalian cells cultured at 37 degreesC and to maximize. . .

CT Check Tags: Human
Fluorescence
*Gene Transfer Techniques
*Genes, Reporter
*Luminescent Proteins: CH, chemistry
Luminescent Proteins: GE, genetics
Point Mutation

L4 ANSWER 9 OF 11 MEDLINE DUPLICATE 3

AB . . . in soil bacteria (A. G. Matthysse, S. Stretton, C. Dandie, N. C. McClure, and A. E. Goodman, FEMS Microbiol. Lett. **145**:87-94, 1996) were assessed by epifluorescence microscopy for use in tagging three marine bacterial species. Expression of **gfp** could be visualized in Vibrio sp. strain S141 cells at uniform levels of intensity from either the lac or the npt-2 promoter, whereas expression of **gfp** could be visualized in Psychrobacter sp. strain SW5H cells at various levels of intensity only from the npt-2 promoter. Green fluorescent protein (**GFP**) fluorescence was not detected in the third species, Pseudoalteromonas sp. strain S91, when the **gfp** gene was expressed from either promoter. A new mini-Tn10-kan-**gfp** transposon was constructed to investigate further the possibilities of fluorescence tagging of marine bacteria. Insertion of mini-Tn10-kan-**gfp** generated random stable **mutants** at high frequencies with all three marine species. With this transposon, strongly and weakly expressed S91 promoters were isolated. Visualization of **GFP** by epifluorescence microscopy was markedly reduced when S91 (mini-Tn10-kan-**gfp**) cells were grown in rich medium compared to that when cells were grown in minimal medium. Mini-Tn10-kan-**gfp** was used to create an S91 chitinase-negative, **GFP**-positive **mutant**. Expression of the chi-**gfp** fusion was induced in cells exposed to N'-acetylglucosamine or attached to chitin particles. By laser scanning confocal microscopy, biofilms consisting of microcolonies of chi-negative, **GFP**+ S91 cells were found to be localized several microns from a natural chitin substratum. Tagging bacterial strains with **GFP** enables visualization of, as well as monitoring of gene expression in, living single cells in situ and in real time.

L4 ANSWER 10 OF 11 BIOTECHDS COPYRIGHT 2002 DERWENT INFORMATION LTD

TI New modified **Aequorea** green fluorescent polypeptides;

. green fluorescent [redacted] engineering for altered ex[redacted]n and/or emission spectrum, for use as a reporter gene

AB A new fluorescent product derived from a modified **Aequorea** wild-type green fluorescent protein (**GFP**) shows a different excitation and/or emission spectrum from the wild-type product after oxidation and cyclization of amino acid residues 65-67.. . . be a change in the ratio of 2 main excitation peaks, a shorter wavelength peak, or enhanced emission. The modified **GFP** may have the following substitutions: Ser-202 by Phe, and Thr-203 by Ile; or Ile-167 by Val or Thr; Ser-65 by Thr, Met-153 by Ala, and Lys-238 by Glu; Tyr-66 by Phe, His or Trp; Tyr-66 by His and Tyr-**145** by Phe; Tyr-66 by Trp, Asn-46 by Ile, Met-153 by Thr, Val-163 by Ala and Asn-212 by Lys; Tyr-66 by Trp, Ile-123 by Val, Tyr-**145** by His, His-148 by Arg, Met-153 by Thr, Val-163 by Ala and Asn-212 by Lys; or Ser-65 by Ala, Cys, Thr, Leu, Val or Ile. The modified **GFP** may be used as a reporter gene to monitor gene transcription, localization of expression of 2 genes, temporal gene expression, activation of gene expression, etc. Visibly distinct colors and/or increased emission intensities make the new **GFPs** useful in tracking differential gene expression. (38pp)

CT **AEQUOREA** SP. RECOMBINANT GREEN FLUORESCENT PROTEIN **MUTANT** PREP., DNA SEQUENCE, PROTEIN ENGINEERING, ALTERED EXCITATION, EMISSION SPECTRUM, FLUORESCENCE, APPL. REPORTER GENE, TRANSCRIPTION, EXPRESSION LOCALIZATION, ETC. TRACKING ANIMAL PROTEIN SEQUENCE GENE CLONING **MUTAGENESIS** (VOL.15, NO.21)

L4 ANSWER 11 OF 11 MEDLINE DUPLICATE 4

AB BACKGROUND: Variants of the green fluorescent protein (**GFP**) with different colors would be very useful for simultaneous comparisons of multiple protein fates, developmental lineages and gene expression levels. The simplest way to shift the emission color of **GFP** is to substitute histidine or tryptophan for the tyrosine in the chromophore, but such blue-shifted point **mutants** are only dimly fluorescent. The longest wavelengths previously reported for the excitation and emission peaks of **GFP mutants** are 488 and 511 nm, respectively. RESULTS: Additional substitutions, mainly in residues **145**-163, have improved the brightness of the blue-shifted **GFP mutants** with histidine and tryptophan in place of tyrosine 66. Separate **mutations** have pushed the excitation and emission peaks of the most red-shifted **mutant** to 504 and 514 nm, respectively. At least three different colors of **GFP mutants** can now be cleanly distinguished from each other under the microscope, using appropriate filter sets. A fusion protein consisting of. . . proteolytic cleavage of the linker between the two domains. CONCLUSIONS: Our results demonstrate that the production of more and better **GFP** variants is possible and worthwhile. The production of such variants facilitates multicolor imaging of differential gene expression, protein localization or cell fate. Fusions between **mutants** of different colors may be useful substrates for the continuous in situ assay of proteases. Demonstration of energy transfer between **GFP** variants is an important step towards a general method for monitoring the mutual association of fusion proteins.

CT . . . Cell Line, Transformed
Color
DNA Primers
Energy Transfer
Fluorescence
Luminescent Proteins: CH, chemistry
*Luminescent Proteins: GE, genetics
Molecular Sequence Data
Mutagenesis
Protein Engineering
Structure-Activity Relationship

=> s l1 and 236
L5 27 L1 AND 236

=> s l3 and l5

L6 0 L3 AND L5

=> dup rem l5
PROCESSING COMPLETED FOR L5
L7 6 DUP REM L5 (21 DUPLICATES REMOVED)

=> d 1-6

L7 ANSWER 1 OF 6 MEDLINE DUPLICATE 1
AN 2001496028 MEDLINE
DN 21429750 PubMed ID: 11543664
TI Properties of two EBV Mta nuclear export signal sequences.
AU Chen L; Liao G; Fujimuro M; Semmes O J; Hayward S D
CS Molecular Virology Laboratories, Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, 1650 Orleans Street, Baltimore, Maryland 21231, USA.
NC RO1 CA30356 (NCI)
RO1 CA76595 (NCI)
SO VIROLOGY, (2001 Sep 15) 288 (1) 119-28.
Journal code: XEA; 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200110
ED Entered STN: 20010910
Last Updated on STN: 20011015
Entered Medline: 20011011

L7 ANSWER 2 OF 6 MEDLINE DUPLICATE 2
AN 2000209446 MEDLINE
DN 20209446 PubMed ID: 10744759
TI Additional N-glycosylation and its impact on the folding of intestinal lactase-phlorizin hydrolase.
AU Jacob R; Weiner J R; Stadge S; Naim H Y
CS Department of Physiological Chemistry, School of Veterinary Medicine Hannover, D-30559 Hannover, Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Apr 7) 275 (14) 10630-7.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200005
ED Entered STN: 20000518
Last Updated on STN: 20000518
Entered Medline: 20000508

L7 ANSWER 3 OF 6 SCISEARCH COPYRIGHT 2002 ISI (R)
AN 2000:783103 SCISEARCH
GA The Genuine Article (R) Number: 362TR
TI A half-type ABC transporter TAPL is highly conserved between rodent and man, and the human gene is not responsive to interferon-gamma in contrast to TAP1 and TAP2
AU Kobayashi A; Kasano M; Maeda T; Hori S; Motojima K; Suzuki M; Fujiwara T; Takahashi E; Yabe T; Tanaka K; Kasahara M; Yamaguchi Y; Maeda M (Reprint)
CS OSAKA UNIV, GRAD SCH PHARMACEUT SCI, BIOCHEM & MOL BIOL LAB, 2-2 YAMADAOKA, SUITA, OSAKA 5650871, JAPAN (Reprint); OSAKA UNIV, GRAD SCH PHARMACEUT SCI, BIOCHEM & MOL BIOL LAB, SUITA, OSAKA 5650871, JAPAN; TOHO UNIV, SCH PHARMACEUT SCI, DEPT BIOCHEM, CHIBA 2748510, JAPAN; OTSUKA PHARMACEUT CO LTD, OTSUKA GEN RES INST, KAWAUCHI, TOKUSHIMA 77101, JAPAN; JAPANESE RED CROSS, CENT BLOOD CTR, DEPT RES, SHIBUYA KU, TOKYO 1500012, JAPAN; TOKYO METROPOLITAN INST MED SCI, BUNKYO KU, TOKYO 1130021, JAPAN; GRAD UNIV ADV STUDIES, DEPT BIOSCI, HAYAMA 2400193, JAPAN
CYA JAPAN
SO JOURNAL OF BIOCHEMISTRY, (OCT 2000) Vol. 128, No. 4, pp. 711-718.
Publisher: JAPANESE BIOCHEMICAL SOC, ISHIKAWA BLDG-3F, 25-16 HONGO-5-CHOME, BUNKYO-KU, TOKYO 113, JAPAN.
ISSN: 0021-924X.

DT Article; Journal
FS LIFE
LA English
REC Reference Count: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L7 ANSWER 4 OF 6 MEDLINE DUPLICATE 3
AN 1999223478 MEDLINE
DN 99223478 PubMed ID: 10206973
TI Dimerization of the calcium-sensing receptor occurs within the extracellular domain and is eliminated by Cys --> Ser **mutations** at Cys101 and Cys236.
AU Pace A J; Gama L; Breitwieser G E
CS Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.
NC DK-44484 (NIDDK)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Apr 23) 274 (17) 11629-34.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199905
ED Entered STN: 19990601
Last Updated on STN: 20000303
Entered Medline: 19990520

L7 ANSWER 5 OF 6 BIOTECHDS COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 2000-00899 BIOTECHDS
TI Regulated expression of green fluorescent protein under the control of Aureobasidium pullulans xylanase gene xynA;
potential use as marker for monitoring A. pullulans population, and identifying transcriptional control elements of the endo-1,4-beta-D-xylanase gene
AU Vanden Wymelenberg A; Cullen D; Spear R; *Andrews J
CS USDA; Univ.Wisconsin
LO Department of Plant Pathology, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706, USA.
Email: jha@plantpath.wisc.edu
SO FEMS Microbiol.Lett.; (1999) 181, 2, 205-09
CODEN: FMLED7 ISSN: 0378-1097
DT Journal
LA English

L7 ANSWER 6 OF 6 BIOTECHDS COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 1997-10820 BIOTECHDS
TI New humanized gene for green fluorescent protein and related vectors and recombinant host cells;
with human codon usage, in an adeno virus, adeno-associated virus or retro virus vector, for use as a reporter gene in gene marking, gene therapy, fluorescence-activated cell sorting, etc.
AU Zolotukhin S; Muzyczka N; Hauswirth W W
PA Univ.Florida-Res.Found.
LO Gainesville, FL, USA.
PI WO 9726333 24 Jul 1997
AI WO 1997-US755 17 Jan 1997
PRAI US 1996-588201 18 Jan 1996
DT Patent
LA English
OS WPI: 1997-385337 [35]

=> d 1-6 kwic

L7 ANSWER 1 OF 6 MEDLINE DUPLICATE 1
AB . . . shuttles between the nucleus and cytoplasm. Mta contains a recognized leucine-rich, putative nuclear export signal (NES) between aa 227 and **236**. Deletion of this signal sequence eliminated shuttling, while **mutation** of the core LXL motif in the putative

NES diminished but did [redacted]olish the ability of Mta to [redacted]le from donor to recipient cells in a heterokaryon assay. A double **mutation** of the LXL motif plus an upstream VTL motif eliminated shuttling, suggesting that Mta may have two NES motifs. In confirmation of this, transfer of either the sequence encoding the leucine-rich aa 227-**236** motif or that encoding the adjacent hydrophobic aa 218-227 sequence to a **GFP**-NLS-pyruvate kinase reporter protein conferred the property of cytoplasmic accumulation onto the heterologous protein. Cytoplasmic accumulation of both the aa 225-237. . . 218-227 containing reporters was minimal in the presence of the inhibitor leptomycin B, indicating that both motifs mediated Crm-1-dependent export. **Mutations** in the NES signal sequences abolished the ability of Mta to mediate cytoplasmic accumulation of BALF2 replication gene transcripts. This included **mutation** of the LXL motif which still showed cytoplasmic shuttling, suggesting that the NES **mutations** might have additional effects on Mta function. Wild-type Mta co-immunoprecipitated with the splicing factor SC35 and colocalized with SC35 in transfected cells, modifying endogenous SC35 distribution within the nucleus to give more intense, rounded spots. Interestingly, the NES **mutant** proteins appeared to have altered interactions with the splicing complex, binding more tightly to SC35 in co-immunoprecipitation assays. These observations. . .

L7 ANSWER 2 OF 6 MEDLINE DUPLICATE 2

AB . . . membrane form LPHbeta(final). Pro-LPH is associated through homologous domain IV with the membrane through a transmembrane domain. A truncation of **236** amino acids at the COOH terminus of domain IV (denoted LAC236) does not significantly influence the transport competence of the generated **mutant** LPH1646MACT (Panzer, P., Preuss, U., Joberty, G., and Naim, H. Y. (1998) J. Biol. Chem. 273, 13861-13869), strongly suggesting that. . . into LAC236. Transient expression of the cDNA constructs in COS-1 cells confirm glycosylation of the introduced sites. The N-glycosyl pro-LPH **mutants** are transported to the Golgi apparatus at substantially reduced rates as compared with wild-type pro-LPH. Alterations in LAC236 appear to. . . stable dimeric trypsin-resistant pro-LPH forms. Individual expression of chimeras containing LAC236, the transmembrane domain and cytoplasmic tail of pro-LPH and **GFP** as a reporter gene (denoted LAC236-**GFP**) lends strong support to this view: while LAC236-**GFP** is capable of forming dimers per se, its N-glycosyl variants are not. The data strongly suggest that the LAC236 is. . .

CT . . .

Genes, Reporter
Glycosylation
*Glycosylceramidase: CH, chemistry
*Glycosylceramidase: ME, metabolism
*Intestinal Mucosa: EN, enzymology
Luminescent Proteins: GE, genetics
*Microvilli: EN, enzymology
Mutagenesis, Site-Directed
*Protein Folding
Recombinant Fusion Proteins: BI, biosynthesis
Recombinant Proteins: CH, chemistry
Recombinant Proteins: ME, metabolism
Transfection
Trypsin

L7 ANSWER 3 OF 6 SCISEARCH COPYRIGHT 2002 ISI (R)

AB . . . transcribed in various rat tissues [Yamaguchi, Y,, Kasano, M., Terada, T., Sate, R., and Maeda, M. (1999) FEES Lett. 457, 231-**236**]. Primary structures of the human and mouse orthologous counterparts were deduced from cDNAs cloned by means of polymerase chain reaction,. . . TAP1 and TAPE, although TAPL could have diverged from an ancestor of TAP1 or that of TAP1 and TAP2. The TAPL-**GFP** fusion protein transiently expressed in Cos-1 cells was co-localized with PDI, suggesting that TAPL is inserted into endoplasmic reticulum membrane.. . .

ST Author Keywords: ABC transporter; chromosome; **GFP**; interferon-gamma; peptide transport; TAPL

STP KeyWords Plus (R): FLUORESCENCE INSITU HYBRIDIZATION; BARE LYMPHOCYTE

SYNDROME; DNA-BINDING [redacted]; CELLS; **MUTATION**; POLYMERA[redacted]
SYNTHETASE; SEQUENCES; SITE

L7 ANSWER 4 OF 6 MEDLINE DUPLICATE 3
TI Dimerization of the calcium-sensing receptor occurs within the extracellular domain and is eliminated by Cys --> Ser **mutations** at Cys101 and Cys236.
AB . . . reagents. All studies were carried out on the human calcium-sensing receptor tagged at the carboxyl terminus with green fluorescent protein (hCaR-**GFP**) to permit identification and localization of expressed proteins. Truncations containing either the extracellular agonist binding domain plus transmembrane helix 1 (ECD/TMH1-**GFP**) or the transmembrane domain plus the intracellular carboxyl terminus (TMD/carboxyl terminus-**GFP**) were used to identify the dimerization domain. ECD/TMH1-**GFP** was a dimer in the absence of reducing reagents, whereas TMD/carboxyl-terminal **GFP** was a monomer in the absence or presence of reducing agents, suggesting that dimerization occurs via the ECD. To identify the residue(s) involved in dimerization within the ECD, cysteine --> serine point **mutations** were made in residues that are conserved between hCaR and metabotropic glutamate receptors. **Mutations** at positions 60 and 131 were expressed at levels comparable to wild type in HEK 293 cells, had minimal effects on hCaR function, and did not eliminate dimerization, whereas **mutations** at positions 101 and **236** greatly decreased receptor expression and resulted in significant amounts of monomer in the absence of reducing agents. The double point **mutant** hCaR(C101S/C236S)-**GFP** was expressed more robustly than either C101S or C236S and covalent dimerization was eliminated. hCaR(C101S/C236S)-**GFP** had a decreased affinity for extracellular Ca2+ and slower response kinetics upon increases or decreases in agonist concentration. These results. . .
CT . . .
*Cysteine: ME, metabolism
DNA Primers
Dimerization
Disulfides: CH, chemistry
Disulfides: ME, metabolism
Luminescent Proteins: GE, genetics
Luminescent Proteins: ME, metabolism
Point Mutation
Receptors, Cell Surface: CH, chemistry
Receptors, Cell Surface: GE, genetics
*Receptors, Cell Surface: ME, metabolism
Serine: GE, genetics
. . .

L7 ANSWER 5 OF 6 BIOTECHDS COPYRIGHT 2002 DERWENT INFORMATION LTD
AB A **mutant** form of jellyfish cDNA encoding green fluorescent protein (**GFP**) was fused to the promoter of the Aureobasidium pullulans ATCC 62921 endo-1,4-beta-D-xylanase (EC-3.2.1.8) xynA gene, and the expression vector plasmid pxynEGFP was introduced into A. pullulans. In a manner consistent with regulation of the native xynA gene, **GFP** activity was induced by xylose and repressed by glucose. **GFP** fluorescence intensified with increasing proportion of xylose in molar relation of carbon to glucose. **GFP** could be detected in some cells either by fluorescence-activated cell sorting or microscopically at levels of xylose to glucose as low as 0.0001. On solid media, colony fluorescence was not detected below a level of 0.01 xylose. The **GFP** marker may be a useful tool for monitoring populations of A. pullulans in situ and for identifying transcriptional control elements of xynA. An upstream region of xynA was identified that included a putative cre1 binding consensus sequence **236** bp upstream of the translational start codon. (26 ref)

L7 ANSWER 6 OF 6 BIOTECHDS COPYRIGHT 2002 DERWENT INFORMATION LTD
AB A new humanized green fluorescent protein (**GFP**) gene has a specified DNA sequence, encoding a specified protein sequence, or an S65T or Y66H substitution **mutant**, or a **mutant** with FSYGVQ at residues 64-69 replaced with MGYGVL, and/or at least 10-50% of codon

positions containing a [redacted]ized codon, particularly at [redacted] positions 18, 53, 93, 125, 150, 178, 195, 208, **236** and 224, with numerous other specified features for preferred human codon usage. The gene may be linked to control sequences. . . may be present. The vector may be an adeno virus, adeno-associated virus or retro virus vector, and may express enhanced **GFP** or enhanced blue fluorescent protein. The vector may be introduced into a Vero, HeLa, CHO, COS, W138, BHK, HepG2, NIH3T3, RIN, MD cell, A549, PC12, K562 or 293 cell, a primary cell line or a mammal in vivo. The **GFP** gene and vector may be used in gene marking, gene therapy monitoring, cell enrichment by fluorescence-activated cell sorting, and analyzing. . .

=>
Connection closed by remote host